Biological Control **20,** 96–101 (2001)
doi:10.1006/bcon.2000.0894, available online at http://www.idealibrary.com on IDEAL®

Control of Decay of Apple and Citrus Fruits in Semicommercial Tests with *Candida saitoana* and 2-Deoxy-D-glucose

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Received June 29, 1999; accepted November 2, 2000

Efficacy of a combination of *Candida saitoana* and 0.2% 2-deoxy-D-glucose as a treatment for the biological control of postharvest diseases of apple and citrus fruits was determined in semicommercial tests. The combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose was more effective in controlling decay of 'Rome' and 'Empire' apples than *C. saitoana* alone, and the level of control was comparable or superior to that provided by the synthetic fungicide thiabendazole, depending on the variety used. In orange ('Washington' navel, 'Hamlin', and 'Valencia') and 'Eureka' lemon trials, the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose was also superior to *C. saitoana* for controlling decay, and the control level was equal to imazalil, especially on early-season fruits. On late-season 'Washington' navel orange and 'Eureka' lemon fruits, the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose was superior in controlling decay than *C. saitoana* alone, but control was significantly less than with imazalil. These results indicate the reliability and efficacy of the combination of *C. saitoana* and 2-deoxy-D-glucose for the control of postharvest diseases of apple and citrus fruits. Further tests under commercial conditions are needed to confirm these results.

Key Words: **apple fruits; *Botrytis cinerea; Candida saitoana;* citrus fruits; imazalil; *Penicillium expansum; Penicillium digitatum;* thiabendazole; 2-deoxy-D-glucose.**

INTRODUCTION

In recent years, intense research efforts have been devoted to the development of antagonistic microorganisms to control postharvest diseases, and several microbial antagonists have been identified and shown to reduce decay of a wide variety of fruits (Roberts, 1990; Janisiewicz, 1994, 1998; Wilson and Wisniewski, 1994; Filonow *et al.*, 1996; Bull *et al.*, 1997; Chand-Goyal and Spotts, 1997; El Ghaouth *et al.*, 1998). These research efforts have been initiated as a result of the development of resistance by major postharvest pathogens to synthetic fungicides, health and environmental concerns with pesticide disposal and residues on fresh produce, and major financial expenditures required for registration of new fungicides and reregistration of currently used fungicides (Wilson and Wisniewski, 1994). Currently, two antagonistic microorganisms, a yeast, *Candida oleophila* Montrocher, and a bacterium, *Pseudomonas syringae* Van Hall, are commercially available under the trade names Aspire and Bio-Save, respectively.

Although antagonistic yeasts and bacteria have been shown to reduce postharvest diseases, they have not been shown to offer consistent and effective control comparable to that provided by synthetic fungicides (Brown and Chambers, 1996; Wilson *et al.*, 1996; Droby *et al.*, 1998). The future successful use of microbial biocontrol requires reliable performance resulting in an economically sufficient level of disease control. This may be possible through a multifaceted approach that utilizes the additive or synergistic effects of the combination of microbial biocontrol agents and bioactive compounds. In laboratory studies the addition of $CaCl_2$, nitrogenous compounds, or sugar analogs increased the effectiveness of certain antagonists and greatly reduced the populations of yeast and bacterial antagonists required to provide effective control (McLaughlin *et al.*, 1990; Janisiewicz, 1994, 1998; Janisiewicz *et al.*, 1992, 1998; Wisniewski *et al.*, 1995; Droby *et al.*, 1997).

Recently, we have developed a biocontrol product consisting of a combination of an antagonistic yeast, *Candida saitoana* Nakase&Suzuki, and 2-deoxy-D-glucose (Wilson and El Ghaouth, 1997). The mixture combines the antifungal property of 2-deoxy-D-glucose and

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1049-9644/01 $35.00

the biocontrol activity of the antagonist. Laboratory tests on apple and citrus fruits showed that the combination of *C. saitoana* and 2-deoxy-D-glucose was more effective in controlling decay than either the antagonist or the 2-deoxy-D-glucose alone. The combination of *C. saitoana* with 0.2% 2-deoxy-D-glucose was also effective against infections established up to 24 h before treatment (El Ghaouth *et al.*, 2000).

The present study was undertaken to evaluate the efficacy of *C. saitoana* with 2-deoxy-D-glucose as a treatment for the control of postharvest diseases of apple and citrus fruits under upscale tests. Effectiveness under commercial conditions can be predicted only in tests where commercial equipment, natural inoculation, and two or more seasons of tests are employed.

MATERIALS AND METHODS

Reagents, yeast preparations, and fruit materials. 2-Deoxy-D-glucose was obtained from Sigma Chemical Co. (St. Louis, MO). Imazalil (Fungaflor 500EC, 44.6% ai) was purchased from Janssen Pharmaceutical (Titusville, NJ). Thiabendazole 42.28% ai (2-[4-thiazolyl]-1H-benzimidazole) was obtained from GreenChemicals (Winchester, VA). *C. saitoana* was grown at 24°C for 48 h in 2-liter shake-flask cultures of yeast–maltose broth. Yeast cells were pelleted by centrifugation with a Sorval RC-58 centrifuge (DuPont Instruments, Wilmington, DE) at 3000*g* for 20 min at 20°C, resuspended in sterile distilled water, and centrifuged again, and the pellet was used immediately. *C. saitoana* was also grown in nutrient–yeast broth in IF-15 fermentor (15-liter volume; New Brunswick Scientific Co., Edison, NJ). Fermentation conditions were as follows: aeration 0.666 v air/v medium/ min; agitation, 250 rpm; and temperature, 24°C. After 72 h, the culture, which consisted of 6.5% solids, was harvested using a Sharples AS-16V Super centrifuge (Alfa-Laval Separation, Inc., Warminster, PA) and the resulting wet paste was stored at 4°C. One gram of yeast paste was equivalent approximately to 2.1×10^{10} colony-forming units (CFU).

Tree-ripe apple fruits (*Malus domestica* Borkh.) of cultivars 'Empire' and 'Rome' were hand-picked at harvest maturity at the USDA-ARS, Appalachian Fruits Research Station, Kearneysville, West Virginia. Early and late 'Washington' navel oranges (*Citrus sinens* [L.] Osbeck.) and 'Eureka' lemons (*Citrus lemon* [L.] Burm.) grown in the San Joaquin Valley of California were harvested within 2 days before treatment. 'Valencia' and 'Hamlin' oranges grown in Lake Alfred, Florida, were hand-harvested 24 h before treatment. Apples, oranges, and lemons were sorted to remove any with apparent injuries or infections, washed with water on a processing line, and randomly assigned to different treatments.

Semicommercial tests. Experiments were conducted from 1995 to 1997 on semicommercial packing lines at Kearneysville, West Virginia, Lindcove, California, and Lake Alfred, Florida. At Kearneysville, apple fruits ('Rome' and 'Empire') were wounded (3 mm by 5 mm deep) once with a blunted steel finishing nail, placed on a dry dump conveyor-belt, and passed over rotating brushes through a washer operating at 5.8×10^3 N/m^2 to an air drier operating at 32°C. Subsequently, the fruits were sprayed with water; yeast cell suspensions (10^8 CFU/ml) containing 0.2% (w/v) of 2-deoxy-D-glucose at approximately 15 ml/kg of fruits or 600 ppm of thiabendazole (full label rate) using six flat-fan nozzles at a pressure of 9.2×10^2 N/m^2. The fruits were then passed through an air drier to a moving-belt sorting table, where decayed or damaged fruits were removed. Within each experiment, treatments were applied to five replicates of 56 apples and the tests were conducted during three successive seasons. Before the application of each treatment, the rollers and nozzles were washed extensively. Treated 'Rome' and 'Empire' apples were packed in cardboard boxes, stored at 18°C for 2 and 5 weeks, respectively, to mimic consumer's display conditions, and to obtain sufficient disease level. Fruits were evaluated periodically for disease development.

At the packing house in Lindcove, orange and lemon fruits were placed on a dry dump conveyor-belt and passed over rotating brushes through a high-pressure washer operating at 4.3×10^4 N/m^2 where they were washed extensively with water containing 50 μg/ml of sodium hypochlorite at pH 7.2. After being washed, the fruits passed over foam rubber rollers for drying to a moving-belt sorting table, where decayed or damaged fruits were removed. Lemon fruits were submerged for approximately 2 min into a 2400-liter-capacity tank containing a solution of 3% (w/v) sodium carbonate heated to 37°C and then lifted by rollers to a washer with a series of overhead nozzles and then to foam-rubber rollers for drying. In California packing houses, lemon fruits are often treated with sodium carbonate (Na_2CO_3) to improve cleaning and to reduce postharvest decay (Eckert and Eaks, 1989).

Orange fruits bypassed the sodium carbonate treatment and fruits were moved to the washing unit that consisted of nylon brushes for washing and foam-rubber rollers for drying. Dried lemons and oranges were treated with water; yeast cell suspensions (10^8 CFU/ml) containing 0.2% (w/v) of 2-deoxy-D-glucose at the same rate described above or 2000 ppm of imazalil (1-[2-(2, 4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole; label rate in California commercial packinghouses). After treatment, the fruits passed through a high-velocity air drier operating at 32°C, waxed with a water–emulsion storage wax with a 2% solids content, dried by passage through a high-velocity air drier, and dried again by passing through a high-velocity air

drier. All the treatments except imazalil were applied using an on-line overhead spray system installed before the waxer. Rollers and nozzles were washed extensively between application of different treatments. The imazalil treatment was applied in the water–emulsion storage wax using the waxer with an overhead single nozzle that continually moved from side to side (set up number for 1/4 J round spray, Spray Systems Co). Within each experiment, the treatments were 12 replicates of 60–75 'Washington' navel oranges and 10–12 replicates of 110–140 'Eureka' lemons. The tests were conducted over three successive seasons. Treated fruits were packed in commercial cartons and stored for 25 days at 10°C, and the incidence of decay was determined.

At the packing line in Lake Alfred, Florida, fruits were placed on a dry dump conveyor-belt where decayed or damaged fruits were removed and then passed over rotating brushes through a washer and dryer unit similar to that described above. Fruits were treated with water; yeast cell suspensions (10^8 CFU/ml) containing 0.2% (w/v) of 2-deoxy-D-glucose at the same rate described above or 1000 ppm of imazalil label rate used in Florida commercial packing houses) using a controlled-drip applicator that delivered 350 ml of the suspension per minute to fruits rotating on brushes saturated with the treating material. Before the application of each treatment, the rollers and nozzles were washed extensively. After treatment, the fruits were dried by passing through a high-velocity air drier operating at 32°C. Within each experiment, treatments were applied to eight replicates of 50 'Hamlin' and 'Valencia' oranges. Tests were conducted during three successive seasons. 'Hamlin' and 'Valencia' oranges were packed in cardboard boxes and stored for 21 and 28 days, respectively, at 18°C. Fruits were evaluated periodically for disease development.

Statistics. An arcsine-square root transformation of the proportion of decayed fruits was applied to the data from the different trials prior to analyses of variance. Homogeneity of variance for different trials of each experiment was evaluated by Hartley's *F*-Max test at $P = 0.05$. Data from separate trials were combined when statistical analysis determined variances were homogeneous. Duncan's new multiple range test ($P = 0.05$) to separate means was applied to compare treatments.

Fruit colonization by C. saitoana. The effect of 2-deoxy-D-glucose on the survival of *C. saitoana* in apple wounds and on apple surfaces was also determined. 'Rome' apples were wounded once as described above, processed on-line, and treated with a yeast cell suspension (10^8 CFU/ml) containing 0 or 0.2% (w/v) of 2-deoxy-D-glucose as described previously. Treated fruits were stored at 24°C under high relative humidity (95% RH) in enclosed plastic containers. For each treatment, four replicates of five fruits each were arranged in a randomized complete block.

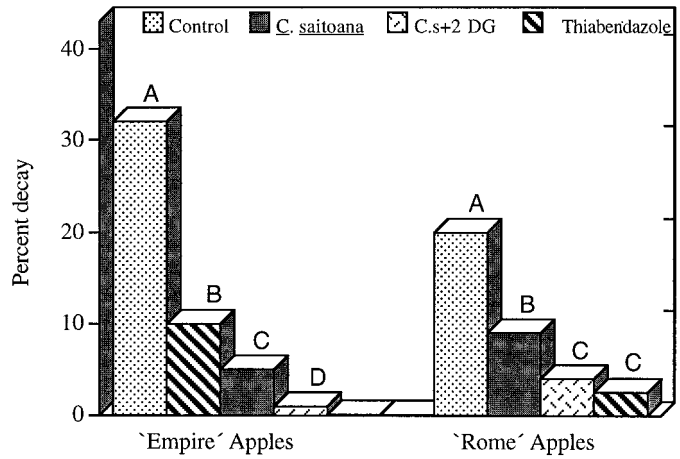

FIG. 1. Effect of the combination of *Candida saitoana* and 0.2% (w/v) 2-deoxy-D-glucose (C.s+2DG) on decay of 'Empire' and 'Rome' apples. The percentage of decay was based on five replicates of 56 fruits each. Incidence of decay on 'Empire' and 'Rome' apples was determined after 14 and 21 days, respectively, of storage at 18°C. Bars within each cultivar with the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

Samples were collected 0 and 21 days after treatment. At each sampling time, tissue containing the wounds was removed with a No. 7 cork borer (6 mm in diameter) from four apples selected randomly from each treatment. The tissue was homogenized in 5 ml of sterile water and vortexed. The selected fruits with removed wounds were placed individually in sterile plastic bags with 200 ml of sterile water and shaken vigorously for 5 min. Tissue homogenate and fruit-wash were dilution-plated in triplicate on a yeast–maltose agar medium and the plates were incubated at 24°C. Colonies were counted after 48 h and the results are expressed as the mean number of colony-forming units per square centimeter.

RESULTS

Semicommercial tests on control of apple and citrus decay. The combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose treatment controlled effectively decay of 'Rome' and 'Empire' apples caused primarily by *Botrytis cinerea* Pers.:Fr. and *Penicillium expansum* Link (Fig. 1). The combination of *C. saitoana* with 0.2% 2-deoxy-D-glucose controlled decay significantly better than *C. saitoana* alone and the water control, and the level of control was similar or superior to the control from thiabendazole treatment, depending on the fruit variety used (Fig. 1). When used alone, *C. saitoana* was significantly superior to the water-treated control in reducing decay. At the end of the storage period, treatment with the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose resulted in 85 to 95% less decay in

TABLE 1

Survival of *Candida saitoana* in Wounds and Surfaces of 'Rome' Apple in the Presence and Absence of 2-Deoxy-D-glucose

Treatment[a]	Storage period (days)	Yeast cell counts (log CFU/cm^2) Surface	Wound
C. saitoana	0	5.47 (±0.10)	5.38 (±0.12)
	21	5.37 (±0.13)	6.73 (±0.15)
C. saitoana + 2-deoxy-D-glucose	0	5.22 (±0.12)	5.41 (±0.14)
	21	5.31 (±0.11)	6.61 (±0.16)

[a] Wounded apples were treated with a *C. saitoana* cell suspension (10^8 CFU/ml) containing 0 or 0.2% (w/v) 2-deoxy-D-glucose. Values in parentheses are standard errors of the mean.

comparison to the control, whereas a reduction of 45 to 85% was observed among apple fruits treated with *C. saitoana* alone. The thiabendazole treatment of 'Empire' and 'Rome' apples reduced decay by 69 and 88%, respectively. In apple wounds and on apple surfaces, the growth of *C. saitoana* did not appear to be affected by the addition of 2-deoxy-D-glucose (Table 1). After 21 days, *C. saitoana* populations in apple wounds increased more than 20-fold, whereas they remained unchanged on the fruit surface.

The combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose was also effective in controlling decay of oranges and lemons in trials at Lindcove, California. The best performance was on early-season fruits (Figs. 2 and 3). Green mold caused by *Penicillium digitatum* (Pers.: Fr.) Sacc. was the most prevalent disease, and

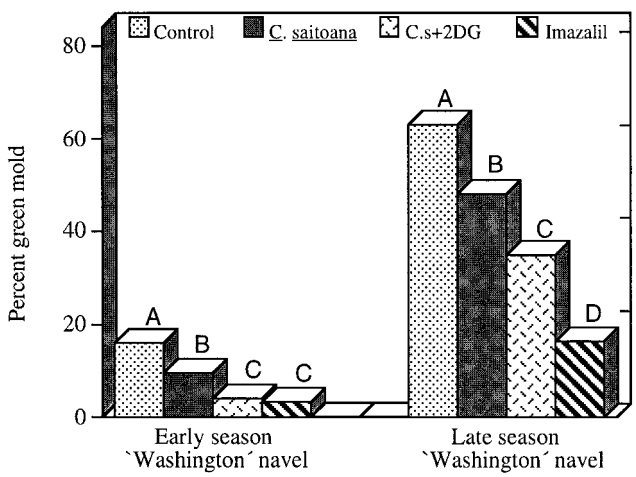

FIG. 2. Effect of the combination of *Candida saitoana* and 0.2% (w/v) 2-deoxy-D-glucose (C.s+2DG) on decay of early- and late-season 'Washington' navel oranges. The percentage of decay was based on 12 replicates of 60–75 fruits each. Incidence of decay was determined after 25 days of storage at 10°C. Bars within each season with the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

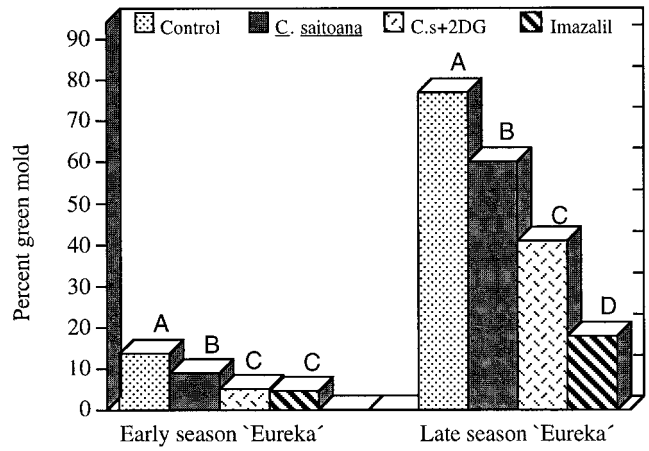

FIG. 3. Effect of the combination of *Candida saitoana* and 0.2% (w/v) 2-deoxy-D-glucose (C.s+2DG) on decay of early- and late-season 'Eureka' lemons. The percentage of decay was based on 10–12 replicates of 110–140 fruits each. Incidence of decay was determined after 25 days of storage at 10°C. Bars within each season with the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

the incidence of blue mold and sour rot caused by *Penicillium italicum* Wehmer and *Geotrichum candidum* Link ex Pers, respectively, was very low. The combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose was superior to *C. saitoana* in controlling decay on early-season oranges and lemons, and the control was comparable to that with 2000 μg/ml imazalil (Figs. 2 and 3). The incidence of decay among early-season oranges and lemons treated with *C. saitoana* was significantly lower than that in the water control, but much higher than in the treatment with the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose or imazalil. After 24 days of storage, the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose reduced decay on early-season oranges and lemons by 75 and 63%, respectively, while a reduction of 67 and 80% was observed among oranges and lemons treated with 2000 μg/ml of imazalil. *C. saitoana*, when used alone on oranges and lemons, resulted in 40% less decay than in the water-treated control.

Incidence of decay was higher in late season than on early season oranges and lemons treated with the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose. On late-season oranges and lemons, the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose provided a level of control superior to that from *C. saitoana* alone, but significantly lower than in the imazalil treatment (Figs. 2 and 3). Treatment of late-season oranges and lemons with the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose resulted in more than 45% less decay than the water-treated control, while the imazalil reduced the incidence of decay by more than 65% (Figs. 2 and 3). The decay incidence following *C. saitoana* treatment alone was approximately 20% less than in the water-treated control.

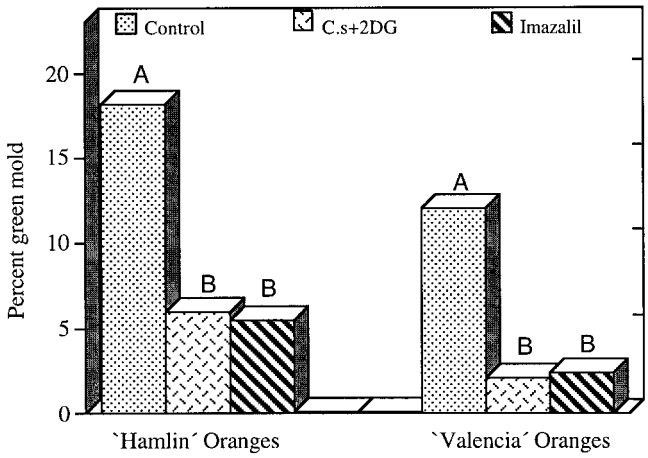

FIG. 4. Effect of the combination of *Candida saitoana* and 0.2% (w/v) 2-deoxy-D-glucose (C.s+2DG) on decay of 'Hamlin' and 'Valencia' oranges. Percentage of decay was based on eight replicates of 50 fruits each. Incidence of decay on 'Hamlin' and 'Valencia' oranges was determined after 21 and 28 days, respectively, of storage at 18°C. Bars within each cultivar with the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

Improved efficacy by the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose in controlling decay of oranges, compared to water control, was observed in semicommercial tests conducted in Florida (Fig. 4). The combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose controlled green mold of 'Hamlin' and 'Valencia' oranges caused by *P. digitatum*, as effectively as imazalil. At the end of the storage period, the incidence of decay among 'Hamlin' and 'Valencia' oranges treated with the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose was 6 and 2.1%, respectively, levels similar to those obtained with imazalil (Fig. 4).

DISCUSSION

The combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose reduced the incidence of decay of apple and early-season citrus fruits to a level equivalent to that achieved with the commercial fungicides thiabendazole and imazalil. Control levels equivalent to commercial fungicide treatments were also reported with other antagonistic yeasts when used in combination with low doses of fungicides (Brown and Chambers, 1996; Droby *et al.*, 1998; Sugar and Spotts, 1999). On citrus fruits, the combination of thiabendazole (200 ppm) with either *C. oleophila* or *Pichia guilliermondii* Wickerham was shown to provide control comparable to that from the commercial recommended dose of thiabendazole (2000 ppm) and imazalil (2000 ppm) (Droby *et al.*, 1993, 1998).

In semicommercial trials, the combination of *C. saitoana* with 0.2% 2-deoxy-D-glucose offered significantly superior control to *C. saitoana* alone, thus indicating *an* enhancement of the bicontrol efficacy or activity of *C. saitoana* by the addition of 0.2% 2-deoxy-D-glucose. 2-Deoxy-D-glucose, although inhibitory to some postharvest pathogens (El Ghaouth *et al.*, 1995, 1997), showed no effect on the growth of *C. saitoana* in apple wounds and surfaces. In laboratory studies, several naturally occurring organic and inorganic additives were also shown to increase the performance of selected microbial antagonists (Janisiewicz, 1994; Wisniewski *et al.*, 1995; Chand-Goyal and Spotts, 1997). The addition of $CaCl_2$ enhanced the effectiveness of certain antagonists (McLaughlin *et al.*, 1990; Wisniewski *et al.*, 1995). Enhancement of biocontrol activity of antagonists by nitrogenous (L-asparagine and L-proline) and carbohydrate (2-deoxy-D-glucose) compounds also was reported in apple and pear fruits (Janisiewicz *et al.*, 1992; Janisiewicz, 1994). The addition of sugar analog 2-deoxy-D-glucose was shown to enhance biocontrol of blue mold of apple by *P. syringae* and *Sporobomyces roseus* Kluyver & Niel (Janisiewicz, 1994).

Although the combination of *C. saitoana* with 0.2% 2-deoxy-D-glucose was effective in controlling decay of early-season citrus, its performance declined on late-season fruits. The increased availability of nutrients, especially carbohydrates, in late-season fruits as a result of the biochemical changes associated with ripening may have offset the inhibitory effect of 2-deoxy-D-glucose and the antagonistic activity of *C. saitoana*. Therefore, a reduction in the efficacy of the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose can be anticipated. The inhibitory activity of 2-deoxy-D-glucose with more mature fruits can be reversed with utilizable sugars, in particular glucose (Moore, 1981). Also, currently available microbial antagonists confer only a protectant effect that diminishes with ripening (Roberts, 1990; Smilanick, 1994; Wilson *et al.*, 1996). In citrus fruits, the biocontrol activity of antagonistic yeasts has been negated by the addition of nutrients to the wound site (Droby *et al.*, 1989).

In conclusion, this study demonstrates the reliability and efficacy of the combination of *C. saitoana* and 0.2% 2-deoxy-D-glucose as a viable alternative to synthetic fungicides for the control of some postharvest diseases of apple and early-season citrus fruits. The level of control conferred by the combination of *C. saitoana* with 0.2% 2-deoxy-D-glucose was superior to that by *C. saitoana* and often equivalent to those afforded by the synthetic fungicides thiabendazole and imazalil. The complexity of the mode of action of combined alternatives may make the development of fungicide resistance, a significant problem with fungicides currently used to control postharvest decay (Eckert *et al.*, 1994), more difficult and provide a greater stability and effectiveness than the approach of utilizing a single microbial biocontrol agent. Further studies under commer-

cial conditions are needed to confirm these observations.

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